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Article

Effects of Waxy Types of a Sugarcane Stalk Surface on the Spectral Characteristics of Visible-Shortwave Near Infrared measurement

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Abstract. The precision of spectroscopic methods was frequently affected by the identity of the inhomogeneous materials, especially for direct scanning. This research aimed to investigate the effects of waxy types, naturally found on sugarcane surface, on spectral characteristics. A portable Vis/SWNIR instrument with interactance mode across wavelength of 570-1031 nm was used for direct scanning the cane stalk. Principal component analysis (PCA) was applied to examine the differences in the spectra scanned from 180 samples, including 3 types of waxy type: white, black, and mixed black and white. Seven widespread pretreatments were employed to reduce the effect of the waxy types. Results show that the spectra of the samples with each waxy type was separated in groups and standard normal variate (SNV) pretreatment gave the best results. However, it was not able to eliminate the effect compared to the wax-removed samples. Meanwhile, the standard deviation of absorbance values, at the wavelength of 760, 904 and 970 nm of 3 samples, was used for assessing the repeatability and reproducibility. The samples where the waxy covers were removed provided a lower standard deviation of absorbance values of spectra than the best pretreated spectra that used SNV of the samples that retained its waxy covers by one to six times. Thus, the waxy material on cane surface should be removed before collecting spectra.

Keywords: Sugarcane quality, visible-shortwave near infrared spectroscopy, cane stalk, PCA.

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1. Introduction

Sugarcane originated from New Guinea and their planting techniques were transferred to China, North Africa, Spain, and North and South America in the 7th and 8th centuries [1]. According to the World Crop and Livestock Statistics, published by the Food and Agriculture Organization (FAO), Brazil is the largest sugarcane-producing country followed by India, China and Thailand [2]. In 2016, Thailand was the second largest exporter of sugar with an export capacity of 2.3 billion dollars (World's Top Exports, 2016). At present, sugarcane is an important crop for food and fuel. Sugar is mainly utilized for its sucrose that is used as a sweetener. Bagasse, the biomass residue obtained post sucrose extraction, is utilized as fuel to provide steam for generating electricity.

In Thailand, payment determination is majorly considered according to sugarcane weight and quality. The quality is similarly defined in Australia where it is referred to as commercial cane sugar (CCS) which is derived from soluble solid content (Brix), sugar content that is measured using the property of optical activity which causes polarized light to be rotated (Pol) and the fiber content. CCS is a key parameter in estimating the quantity of sugar that can be extracted from cane stalks. Fiber content is another key parameter that is usually employed for the estimation of sugarcane bagasse. These parameters were very important in harvest management, adoption of precise agriculture techniques, and for the purpose of payment to growers. Furthermore, it provides an estimation of energy output and the obtained bagasse that can be used to provide steam in power plants and, especially, for aiding breeding programmes. However, the conventional method to measure quality requires destructing the cane stalk and is quite time-consuming. Hence, it is very difficult to perform measurements in the field or somewhere else that is not a laboratory.

There are several studies that have reported about the estimation of sugarcane quality using the application of spectroscopic methods [3, 4, 5]. This technology is a method of measuring the quality in food and a rapid analysis of the properties in any materials, such as satsuma mandarin [6], peaches [7], mango [8, 9], chilling peach [10] and longan [11].

Recent studies by Nawi et al. [12, 13, 14] developed the spectroscopic method to predict the parameters of sugarcane quality by directly scanning the sugarcane stalk utilizing a visible-shortwave near infrared (Vis/SWNIR) spectroradiometer. Furthermore, a similar study by Taira et al. [15] developed a non-destructive method for the measurement of sugar content in sugarcane stalks using a portable (Vis/SWNIR) instrument. These studies demonstrated that the spectroscopic method has the potential to evaluate the sugarcane quality in cane stalks. However, while scanning the cane surface directly, the spectra may be influenced by the various colors of the surface. Moreover, Mangesh et al. [16] revealed that the composition of crude wax was alkanes (28.83%), ester (66.26%), fatty acids (4.58%), aldehyde (0.11%), and alcohol (0.22%) which was obtained from gas chromatography-mass spectroscopy (GC-MS). These complements produced prominent peaks during the Fourier transform-infrared spectroscopy (FT-IR) analysis. These exposures indicate that the wax on the sugarcane surface may influence the spectra of visible-shortwave near infrared. Therefore, the accuracy of spectra acquisition should be examined to design the sample preparation before modeling, such as by using the repeatability and reproducibility values [17] or principal component analysis [18]. The PCA is a statistical procedure that uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables [18]. This method was widely used to identify the cultivars of fruits and plants by detecting the differences among the cultivars such as European pear cultivars [19] and the peach variety [18]. Therefore, the PCA method was used to examine the effect of waxy types on the cane surface.

If this consideration can be defined through its ultimate effect, it will be tangible data for eliminating or avoiding the effect of getting low accuracy of the models. Thus, this study was focused on the spectral differences and precision test for spectral characteristics of Vis/SWNIR spectroscopy caused by the different waxy types while measuring the sugarcane quality of cane stalks.

2. Materials and Methods

2.1. Sample Preparation

Wax on the sugarcane surface consists of several compositions [16] which can display itself in multi-color. In this experiment, cane wax was classified into three groups based on its color, namely black, white, and mixed black and white. This classification was set with the assumption that the color of the wax might be the main factor in impacting the spectra acquisition due to the fact that visible wavebands interact with the color. Referring to the physiology of sugarcane growth, height of the stalk is increased through the expansion of the number of internodes during the maturation stage. The internodes at the lower part of the stalk (bottom section) are older than the one at the upper part. Therefore, the three sub-sections have different substances and structures. These general characteristics of sugarcane lead to the practical measurement of the cane quality in the breeding programmes, wherein the process of cane quality measurement is divided by the bottom, middle, and top sections for studying physiology in each cultivar. Thus, the protocol of the experiment included the cane samples of three sub-sections within the different varieties. The samples were obtained by cutting cane stalks 10 cm above the ground. Thereafter, all the leaf was removed. Subsequently, the cane stalk was divided into three sub-sections i.e. top, middle, and bottom, each with a length of 40 cm. Finally, the samples were taken to a laboratory and kept at room temperature of 25±2 ºC for an hour prior to scanning. Each sample was scanned two times. The first scan was performed without preparing the sample (original sample). On the other hand, the second scanning was performed after removing the waxy material on the cane surface (prepared sample). The second scan used the same samples as the first scan.

In this study, the total 180 sugarcane samples were obtained from 60 stalks that were acquired from the Khon Kean Field Crops Research Center (KKFCRC) during the 2016/2017 harvest season. These were used for investigating the differences in spectra caused by the various waxy types including black (60 samples), white (60 samples), and mixed black and white (60 samples) as shown in Fig. 1(a). These samples include 6 varieties (KK3, LK92-11, TP1, TP2, UT-15 and K07-037) grown in Northeast Thailand which were 8-12 months of age.

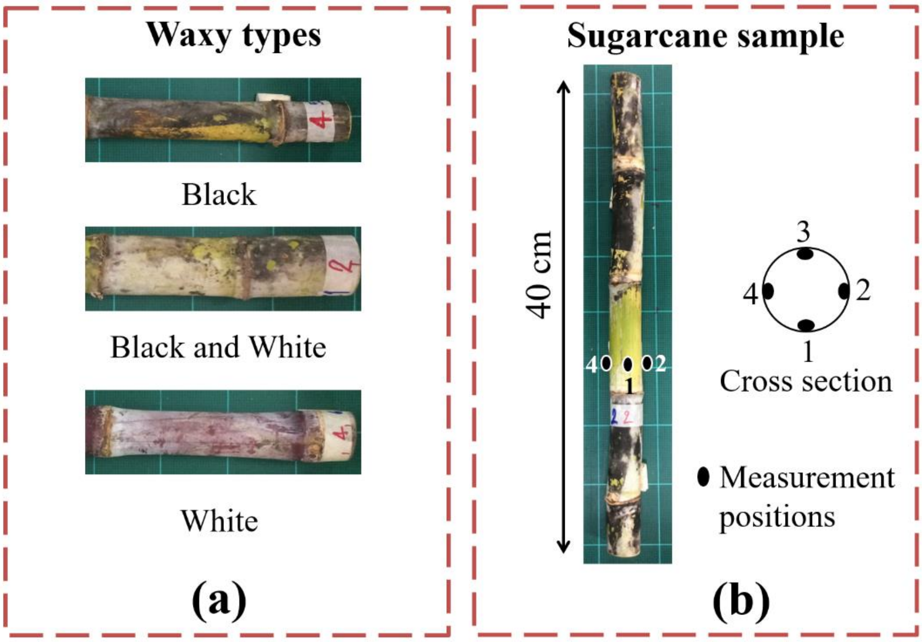

Fig. 1. (a) Waxy types on cane surface (b) measurement position of cane sample.

2.2. Spectral Acquisitions

A portable visible-shortwave near infrared (Vis/SWNIR) spectrometer (HNK Engineering, Hokkaido, Japan) with interactance mode across wavelength of 570-1031 nm at 1 nm interval and integration time of 300 ms was applied for direct scanning on the sugarcane samples in the laboratory, at room temperature of 25±2 ºC. A small halogen lamp was used as a light source and fiber optic was used for signal transmission. A computer using Windows 8 (Microsoft Corporation, Redmond, Washington, 123 United States) was used for storing and processing the spectra data.

Prior to scanning, a standard black sheet (Ethylene-vinyl acetate, EVA) and teflon plate of 2 mm in thickness were scanned as reference materials. Each sample was randomly scanned across the middle part, above a growing point of approximately 20 mm, as the first position. Afterward, the next three scanning positions were performed at 90º, 180º, and 270º, respectively, from the first position until the sample was scanned all around [15] as shown in Fig. 1(b). Finally, the spectrum of each sample was computed by averaging four successive scans.

2.3. Principal Component Analysis and Spectral Data Pretreatment

PCA was applied to examine the prominent differences between waxy types on the cane surface. The PC scores of the samples were calculated by the PCA method using the MATLAB program (MathWorks version 2017, Massachusetts, USA), then plotted between PC1 and PC2 to check the patterns in the samples. If the plot of the PC scores represents the patterns in the samples and are categorized into groups, it may conclude that the waxy types affect the Vis/SWNIR spectra.

Seven widespread pretreatment techniques, including baseline offset, mean centering, standard normal variate (SNV), range normalization, moving average smoothing (21 data points), first derivative (21 segments, 21 gaps), and second derivative (21 segments, 21 gaps), respectively, were employed to investigate whether a preprocessing technique can eliminate or reduce the effect of the wax on spectra scanning. Pretreatment techniques were performed using the MATLAB program (MathWorks version 2017, Massachusetts, USA).

2.4. Repeatability and Reproducibility

In order to examine the effect of waxy cover on the precision of scanning, the repeatability and reproducibility of the spectral data were determined. Three sugarcane samples were randomly selected from the 180 sugarcane samples. Subsequently, they were scanned at only the first position (see in Fig. 1(b)). The reproducibility of spectral data was calculated as the standard deviation of absorbance values wherein the sample was re-loaded and re-scanned 10 separate times [17]. Meanwhile, the repeatability of spectral data was calculated as the standard deviation of absorbance values where the sample was only re-scanned 10 separate times without re-loading. The absorbance values at any wavelength, which presented the obvious peaks and major changes when the scanning conditions and sample conditions were varied, were used to calculate the repeatability and reproducibility [17].

Moreover, to check whether the algorithm of pretreatment techniques can eliminate or reduce the effect of waxy types, the repeatability and reproducibility of the Vis/SWNIR spectra that were administered the seven widespread pretreatment techniques were also calculated. The repeatability and reproducibility of the pretreated spectra were compared to the raw spectra.

3. Results and Discussion

3.1. Spectral Differences Caused by the Various Waxy Types

To investigate the impact of different sugarcane varieties, the raw spectra were analyzed using PCA. The purpose was to find whether the different varieties would separate the scatter plot between PC1 and PC2. Figure 2 shows that the raw spectra were not categorized into groups, and the six varieties were scattered.

Figure 3 shows the spectra scanned from the original sample. To investigate the differences of spectra that might be affected by waxy types (black; black and white; and white), a PCA method was used to derive the first two components (PC1 and PC2) from the spectral data. Figures 4(a) and 4(b) demonstrate the scores plot of PC1 versus PC2 of different waxy types obtained from the raw spectra for the Vis/SWNIR spectra and SWNIR spectra, respectively. The whole wavelength as well as the SWNIR wavelength give the same results. The sugarcane samples were strongly categorized into three groups. These results indicated that the main variance in spectra was produced from the various waxy types. This characteristic of the spectra would result in low repeatability and reproducibility of prediction and will be a critical issue while developing a robust model [19] since all types of waxy materials can be found on one cane stalk. The spectra scanned from the same cane stalk might differ across a wide range.

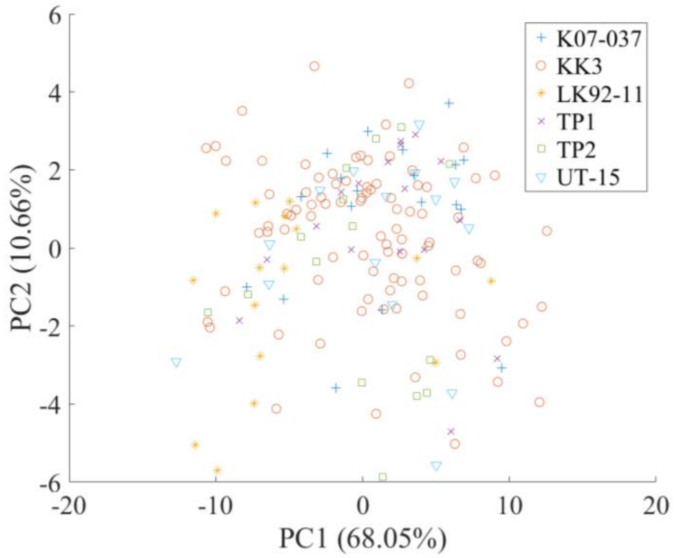

Fig. 2. Scatter plot of PC1 versus PC2 of different sugarcane varieties generated from raw spectra of Vis/SWNIR spectra using PCA.

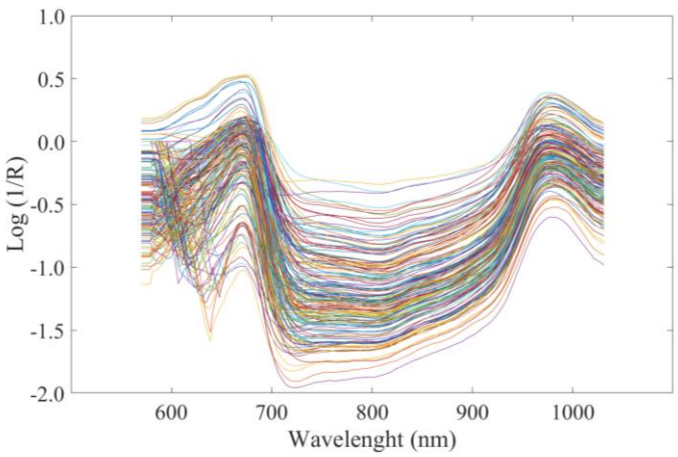

Fig. 3. Raw Vis/SWNIR spectra of cane stalks.

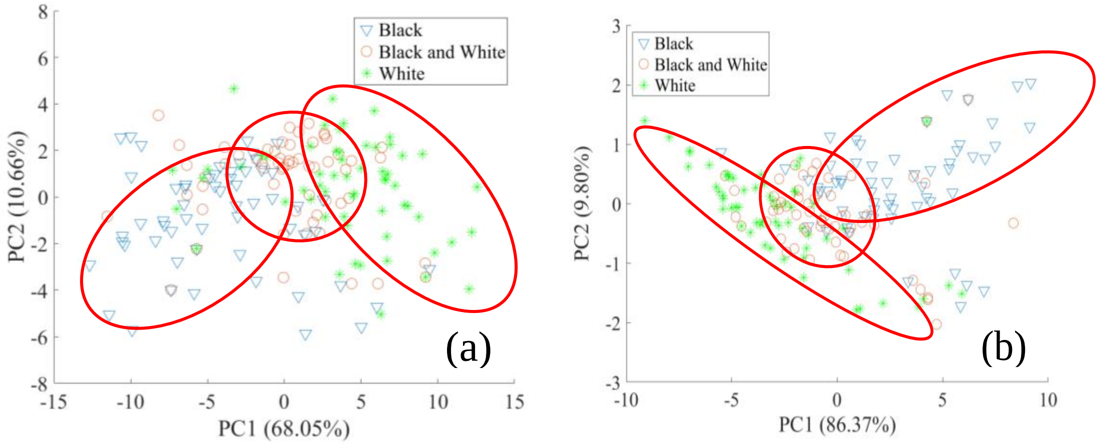

Fig. 4. Scores plot of PC1 versus PC2 obtained from PCA models of different waxy types obtained from raw spectra of (a) Vis/SWNIR spectra; and (b) SWNIR spectra.

Seven spectra pretreatments were applied to check the possibility of reducing the effect of waxy materials on the acquired spectrum with an aim to increase repeatability and reproducibility of prediction. Figures 5(a), (b), (c), (d), (e), (f), and (g) show the scatter plot between PC1 and PC2 of three waxy types built from the pretreated spectra of baseline offset, mean centering, standard normal variate (SNV), range normalization, moving average smoothing, first derivative and second derivative, respectively. The SNV pretreatment turned out to be the best pretreatment in reducing the effect of the covering waxy material because the scatter plot between PC1 and PC2 of three waxy types was slightly separated. It means that SNV helps to eliminate some impact of the wax that covers the cane surface. However, a separation between black and white waxy material still be found. Consequently, the necessity of preparation of the cane samples was brought into consideration. The spectra of the same cane samples were taken again after physically removing the waxy material. Figure 6 shows the scatter plot between PC1 and PC2 for the PCA model built using raw Vis/SWNIR spectra of the prepared samples. The spectra were not categorized into groups depending on their original waxy type.

3.2. Repeatability and Reproducibility of Spectral Data

Repeatability and reproducibility were conducted as a precision test for spectral characteristics. Figures 7(a) and 7(b) represented the raw Vis/SWNIR spectra (571-1031 nm) of sample 3 using re-loaded scanning with the original sample and prepared sample, respectively. The obvious peaks were at around 670, 904, 760 and 970 nm, which relate to chlorophyll [20], carbohydrates [21], 3rd overtone of the O-H stretching mode, and 2nd overtone of the O-H stretching mode [22], respectively. These wavelengths were used to calculate both repeatability and reproducibility of spectra because they were the obvious peaks and were related to the carbohydrates (fructose, glucose, and lactose) [21] and water (H_2O) [22] which are the main substances in sugarcane juice. In addition, these wavelengths showed the greatest change when the scanning conditions and sample compositions were differed. As seen in Fig. 7(a), the spectra were widely offset for the original sample's spectra. Meanwhile, Fig. 7(b) had a narrow offset for the prepared sample's spectra. Therefore, the wax on the cane surface influenced the accuracy of the spectra. This illustrated that the prepared sample gave better reproducibility than the original sample because it was not influenced by the waxy cover.

Table 1 shows the repeatability and reproducibility of absorption values at wavelengths of 760, 904 and 970 nm. Repeatability and reproducibility of spectral data state the precision of the instrument. The absorbance should be the same value when the sample is scanned at the same point. A good repeatability and reproducibility of scanning should present low standard deviation and the spectral shape should be similar.

The mean of absorption and the standard deviation of the observed wavelength in the case of reproducibility of the original sample and the prepared sample were higher than repeatability. This is a typical incidence for the measurement of inhomogeneous material. The mean absorption value of both repeatability and reproducibility for the original samples were almost the same as prepared samples. However, the standard deviations of the prepared samples were less than the original sample around two to twenty times. These results indicated that the waxy materials affect the accuracy of the spectra. The standard deviation of absorbance value of the original samples was higher because the absorption value at the last scanning differs from the absorption value at the first scanning due to the loss of some part of wax material during the duplicate scanning. That is why the sample prepared by removing waxy material can decrease the standard deviation of absorbance and increased the precision of the instrument. This result is similar to the ones discussed by Nawi et al. [14].

The repeatability and reproducibility of raw spectra for original samples pretreated by SNV were less than the non-preprocessed spectra (Table 2). But repeatability and reproducibility of the prepared samples were also less than the SNV spectra by one to six times. Therefore, this demonstrated that the prepared sample also gave better repeatability and reproducibility than the pretreated spectra of the original samples.

These will stand as critical issues for model development. Therefore, to avoid the differences among the waxy types and getting poor repeatability and reproducibility, the sample should be prepared by removing the waxy material on the cane surface before scanning.

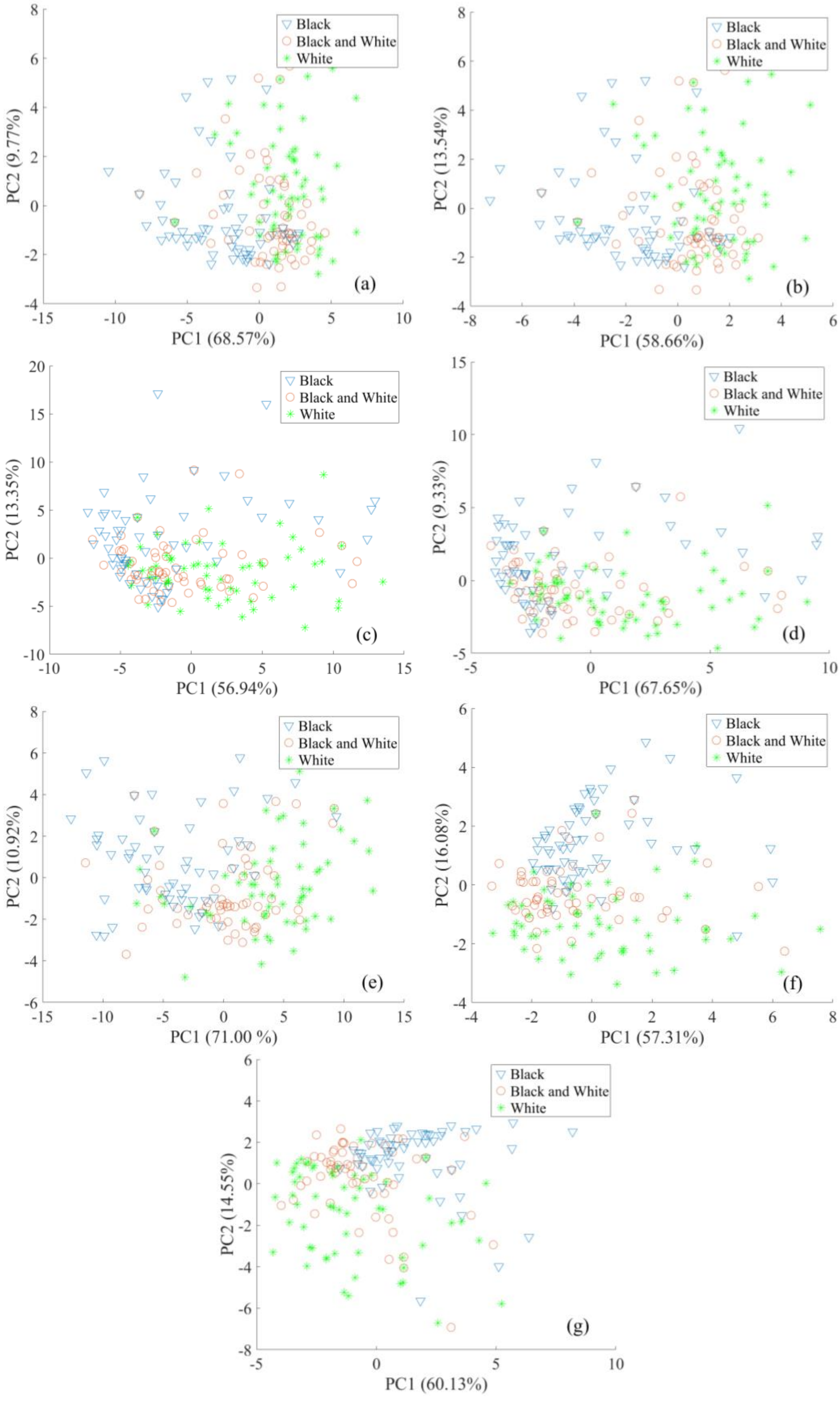

Fig. 5. Scores plot of PC1 vs PC2 built with Vis/SWNIR spectra of different waxy types from (a) baseline offset; (b) mean centering; (c) standard normal variate; (d) range normalization; (e) moving average smoothing; (f) first derivative; (g) second derivative.

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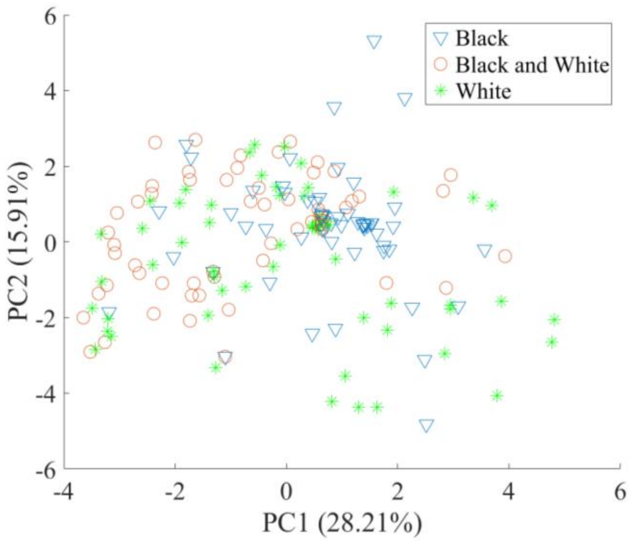

Fig. 6. Scores plot of PC1 versus PC2 for the PCA model built using raw Vis/SWNIR spectra of the prepared samples.

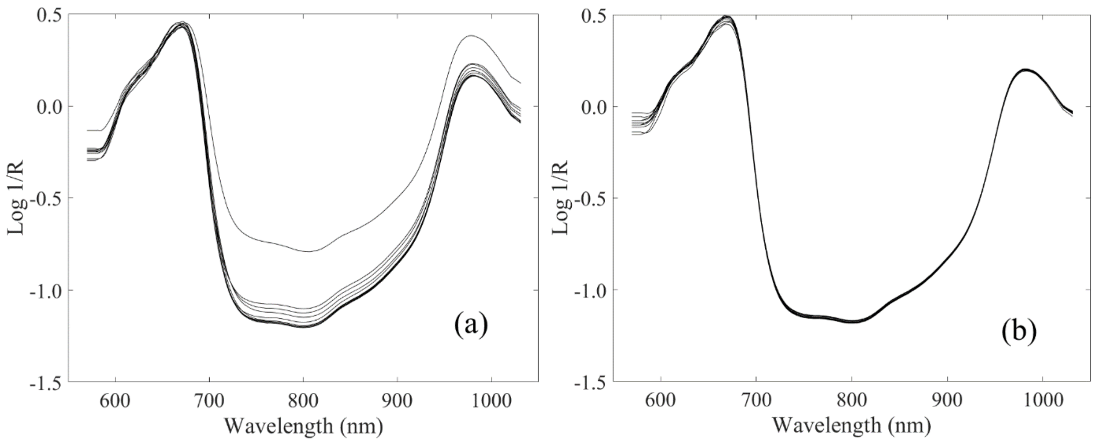

Fig. 7. Raw Vis/SWNIR spectra of sample 3 scanned by re-loading for (a) original sample and (b) prepared sample.

Table 1. Repeatability and reproducibility of absorption values of cane stalks at 760, 904 and 970 nm for raw Vis/SWNIR spectra.

		Original sample				Prepared sample			
		Repeatability		Reproducibility		Repeatability		Reproducibility	
No.	Absorption value	Mean of absorption	Repeat-ability	Mean of absorption	Reprod-ucibility	Mean of absorption	Repeat-ability	Mean of absorption	Reprod-ucibility
1	At 760 nm	-1.241	0.0158	-1.075	0.0372	-1.220	0.0047	-1.069	0.0192
	At 904 nm	-0.867	0.0180	-0.694	0.0281	-0.854	0.0037	-0.684	0.0158
	At 960 nm	0.142	0.0171	0.336	0.0228	0.157	0.0054	0.355	0.0096
	Average	-0.656	0.0170	-0.478	0.0294	-0.639	0.0046	-0.466	0.0149
2	At 760 nm	-1.239	0.0147	-1.131	0.0561	-1.225	0.0079	-1.113	0.0058
	At 904 nm	-0.887	0.0159	-0.777	0.0476	-0.876	0.0073	-0.761	0.0048
	At 960 nm	0.124	0.0131	0.229	0.0312	0.145	0.0082	0.259	0.0053
	Average	-0.668	0.0146	-0.560	0.0450	-0.652	0.0078	-0.538	0.0053
3	At 760 nm	-1.207	0.0186	-1.104	0.1329	-1.186	0.0021	-1.148	0.0065
	At 904 nm	-0.847	0.0165	-0.773	0.1091	-0.824	0.0017	-0.807	0.0048
	At 960 nm	0.136	0.0142	0.173	0.0723	0.171	0.0036	0.161	0.0045
	Average	-0.639	0.0164	-0.568	0.1048	-0.613	0.0024	-0.598	0.0052

No.: sample numbers.

Table 2. Repeatability and reproducibility of absorption values of cane stalks at 760, 904 and 970 nm pretreated by SNV.

		Original			
		Repeatability		Reproducibility	
No.	Absorption value	Mean of absorption	Repeatability	Mean of absorption	Reproducibility
1	At 760 nm	-1.153	0.0024	-1.158	0.0062
	At 904 nm	-0.445	0.0049	-0.443	0.0193
	At 960 nm	1.467	0.0182	1.492	0.0444
	Average	-0.043	0.0085	-0.036	0.0233
2	At 760 nm	-1.150	0.0041	-1.140	0.0078
	At 904 nm	-0.486	0.0098	-0.442	0.0170
	At 960 nm	1.423	0.0346	1.542	0.0415
	Average	-0.071	0.0162	-0.013	0.0221
3	At 760 nm	-1.180	0.0028	-1.190	0.0069
	At 904 nm	-0.569	0.0084	-0.592	0.0117
	At 960 nm	1.102	0.0223	1.121	0.0753
	Average	-0.215	0.0112	-0.220	0.0313

No.: sample numbers.

4. Conclusion

PCA was conducted to investigate the differences in the spectra of cane stalks caused by three types of waxy material. Referring to PCA results, the spectra of the samples were categorized into three groups depending on the waxy types. Comparing the seven spectral pretreatments, SNV pretreatment gave the best results but was not able to completely eliminate the effect. Thus, the preparation of the cane samples was performed by removing waxy material of cane surface. The spectra of wax-removed samples were not categorized in three groups.

Moreover, to assess the effects of the waxy types, repeatability and reproducibility of the spectral data was calculated. The absorbance values at the wavelength of 760, 904 and 970 nm, respectively, were used. The raw spectra of wax-removed samples provided a lower standard deviation of the absorbance values of the spectra than the best pretreated spectra using standard normal variate (SNV) of the original samples by one to six times.

This research shows that the various waxy types affected the spectral differences and spectral characteristics of the Vis/SWNIR spectra of cane stalks which cannot be eliminated by pretreatment techniques. Therefore, prior to scanning, the waxy material should be removed from the cane surface for maximum accuracy in the spectral data.

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Appendix

CCS	=	commercial cane sugar
Vis/SWNIR	=	visible-shortwave near infrared
PCA	=	principle component analysis
SNV	=	standard normal variate
SWNIR	=	shortwave near infrared